REMARKS

Applicants have amended the claims to more particularly define the invention taking into consideration the outstanding Official Action and the interview with Examiner Winkler, the Examiner in charge of this application. Applicants wish to thank the Examiner for the courtesy of the interview during which the distinguishing features of the claimed invention were discussed. While no agreement was reached concerning the patentability of the claimed invention over the prior art, the distinguishing features were discussed and the Examiner agreed to carefully consider this response.

Claims 16-18 have been withdrawn from consideration as being directed to a method of making the novel support used in the kit of the present invention on the grounds that these claims relate to a patentably distinct invention. The reasons for this is said that in this instance, prA coated plates can be made by using a materially different coupling agent therefore the product as claimed can be made by a materially different process that results in the same structure. This statement is specifically traversed and it is most respectfully requested that the restriction requirement be withdrawn.

As previously pointed out, the improved performances obtained with the RT assay kits of the invention is demonstrated in Table 5. HIV-1 RT titration curves were obtained with the previously known Lenti RT kit from Cavidi Tech AB and with an assay kit of the present invention. The OD values can be plotted against the RT enzyme concentration and fitted to a straight line using least squares fit. The k values in Table 6 are the slopes of the calculated straight lines and are a measure of the sensitivity of the assay. It can be seen that the assay kits of the invention have k values that are an order of magnitude higher. In practical terms this translates to useful measurements from samples containing a much lower concentration of HIV-1 reverse transcriptase and/or to time-saving. Shorter reverse transcriptase assay times and/or a shorter alkaline phosphatase reading time means shorter turn-around time for users of the kits of the present invention.

As stated at page 8 of Applicants' specification, the procedure for producing prA coated microtiter plates from a coupling solution is shown in Table 1 to consist essentially of reacting 1-methylimidazole at a pH of 6.25 and polyriboadenylic acid. As stated at page 8, line 12, of Applicants's specification, the mechanism of the binding is not known as the procedure does not correspond to methods recommended or suggested by the manufacturer or is explicitly stated in the literature. A possible reaction mechanism involves reactive groups introduced during the manufacturing procedure but other than those grafted on for the known and intended use of the specific surface. Thus, the amendments to the claims now make it absolutely clear that the product obtained depends on the specified coupling agent. This is in specific contrast to the statement in the Official Action on page 2 that a materially different coupling agent may be used to obtain the product of the present invention.

The exemplification and the claims in the present application clearly emphasize that this is not the case and the additional amendments to the claims simply serves to clarify and does not introduce a new issue at this stage in the proceeding. Moreover, Applicants have clearly established that the subject matter of claims 16-18 are involved with the same inventive concept as the remaining claims acted on the merits. In view of the fact that the claims were withdrawn from consideration, Applicants have not amended these claims at the present time, but subject to their reconsideration would be happy to amend the claims using the language "consisting essentially of" which is believed fully and clearly distinguishes the claimed invention over the prior art.

In this regard, the Examiner's comments on page 3 of the Official Action that it is important to note that the claims are drawn to a product of process wherein the process step comprises methylimidazole as written the claims do not exclude the use of other components in the reaction mixture. It is now believed that other components are excluded from the reaction mixture by the present amendment. These components were previously excluded as the reaction must proceed in accordance with the process as described in Applicants' specification to obtain the kit with the results and sensitivity of the presently claimed invention as discussed at the interview. Accordingly, it is most

respectfully requested that the amendment be entered and claims 16-18 be examined along with the elected invention.

Applicants most respectfully submit that all the claims now present in the application are in full compliance with 35 U.S.C. 112 and are clearly patentable over the references of record. A few additional amendments have been made to the claims to place the Markush language in conventional form.

The rejection of claims 1, 9, 10, 12 and 13-15 under 35 U.S.C. 102(b) as being anticipated by Shao et al. has been carefully considered but is most respectfully traversed. As just noted, the claims as previously written and as now presented clearly exclude the use of other coupling agents in the reaction mixture which do not give the results achieved by the present invention. Moreover, Applicants have provided objective evidence indicating that the prA linked to the polystyrene plate of the instant invention is structurally different than the prA bound to the polystyrene plates of the prior art in terms of the results achieved from the assay of the present invention and those of the prior art. This difference is evidenced by the reactivity and sensitivity of the test of the present invention which is at least an order of magnitude greater than that of the prior art. This is due to the process of making and the resulting structure, that is support used in the kit. The limitations of the product by process claims cannot be ignored but must be taken into consideration and clearly establish the patentability of the claimed invention. The rejection as anticipated by Shao et al should be withdrawn.

Applicants' specification, page 4, lines 22-27 states that the closest prior art to the current invention was developed by Ekstrand et al. 1996, and the method is available as RT determination kits from Cavidi Tech AB, Uppsala, Sweden, the assignee of the present application. Further, on page 10, lines 22-24 is stated that the improved performance obtained with the RT assay kits of the invention is demonstrated in Table 5, HIV-1 RT titration curves were obtained with the previously known Lenti RT kit from Cavidi Tech AB and with an assay kit of the present invention. On page 10, lines 26-28 is stated that the k values in Table 6 are the slopes of the calculated straight lines and are a measure of the sensitivity of the assay. It can be seen that the assay

kits of the invention have k values that are an order of magnitude higher. In the Examples the RT assay kit according to the invention is used, and the results are compared with the results obtained with the Lenti RT assay of Cavidi Tech AB, i.e. assay wherein the prior art microtiter plates with covalently bound prA according to Ekstrand et al., were used. As is evident from the Examples, the difference in detection sensitivity between the RT assay of the invention and the Lenti RT assay of Cavidi Tech AB was approximately 400 times in Example 3 and 25 to 30 fold in Example 4.

The Examiner in her "Detailed action" returns many times (on pages 4, 6, 7, 8, 10, 11) to the statement "the Ekstrand et al. reference cited by Shao et al. indicates that standard chemicals were used for coupling of the prA". So far acceptable. In the next sentence the Examiner, however, claims that standard chemical according to the manufacturers description is the combination carboidiimid and methylimidizol. Applicants most respectfully submit that this is not what Ekstrand et al. says! The actual citation from material and methods on page 97 in Ekstrand et al. reads "Various amounts of prA were evaluated, and linkage was performed by a standard procedure (16)". The reference 16 cited is Suzuki et al. which the Examiner cites on page 12 (Suzuki et al. (J.Viol, Meth 1993, 44 p 189-198). The procedure described on page 191 in this document is based on the use of a **combination** of hydroxysulfosuccinimide and carbodiimide hydrochloride (EDC). This is thus the closest prior art used by the entire three cited authors: Shao et al., Ekstrand et al. and Suzuki et al. All three sources are also mentioned in the international search report.

The Examiner further cites the manufactures description and Rasmussen et al., which is the source referred to in the manufactures description and argues that Rasmussen et al. utilizes the CovaLink NH plates in conjunction with the coupling agent EDC dissolved in 1-methylimidazole for covalent immobilization of double and single stranded DNA on a CovaLink NH plate. "The reference also teaches using various concentrations of EDC and 1-methylimidazole." "Optimizing the conditions such as varying the 1-methylimidazole and condensing agent for binding the target onto the

bottom of the plate would fall within the skill of the ordinary artisan and is suggested by Rasmussen et al."

Applicants agree with the Examiner, this is what might be expected if one where to try to follow the procedure. However, obvious to try is not the standard of obviousness under 35 USC 103(a). Moreover, this is not what happens. What really happened was that Applicants attempts to bind prA according to the teachings of Rasmussen et al. resulted in plates having bound prA with impaired function as template for reverse transcription! The reason for this was not evaluated in detail, but Applicants' results indicate that the method according to Rasmussen et al. is less specific for binding of RNA than of DNA and that the prA was bound also at other position than at the 5' phosphate group. The reactivity of the EDC makes it also likely that it in addition to performing the intended coupling reaction it also to some extent modifies the bases in the prA polymer.

Further experiments revealed that the effect of EDC was clearly harmful and that the optimal immobilized prA template was achieved with 1-methylimidazole only. The efficiency of this binding was a function of both pH and imidazole concentration. EDC at all concentrations was harmful and the ability of prA to act as template decreased with increasing EDC concentration during coupling. EDC treatment of plates with prA that already has been immobilized according to the invention resulted in decreased template function.

Applicants procedure was found useful for coupling of prA to CovaLink plates, Nucleolink strips and Nucleolink plates. Further, it was not useful for immobilization of ssDNA primers, which preferentially are immobilized according to Rasmussen et al. The prA bound according to Applicants' procedure is not affected by a wash of the plate in 1 M NaCl with 0.2 % SDS, which efficiently removes prA unspecifically adsorbed to the plastic.

As already mentioned, the sensitivity of the present assay kit is far better than that of the previous "Ekstrand" assay kit of Cavidi Tech AB, which has been shown in

the specification, but if it will be necessary Applicants have data showing that the EDC technology creates trouble.

Applicants most respectfully submit that all of the claims now present in the application are in full compliance with 35 USC 112 and are clearly patentable over the references of record. Accordingly, it is most respectfully requested that this rejection be withdrawn in view of the above clarification.

For the above reasons, the rejection of claims 1, 9, 10, and claims 12-15 under 35 U.S.C. 102(b) as being unpatentable over Shao et al. has been carefully considered but is most respectfully traversed. As previously noted In this regard, Applicants again wish to direct the Examiner's attention to MPEP § 2131 which states that to anticipate a claim, the reference must teach every element of the claim.

> "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). "The identical invention must be shown in as complete detail as is contained in the ... claim." *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed Cir. 1989). The elements must be arranged as required by the claim, but this is not an *ipsissimis verbis* test, i.e., identity of terminology is not required. *In re Bond*, 910 F.2d 831, 15 USPQ2d 1566 (Fed.Cir. 1990).
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> *Akzo N.V. v. International Trade Comm'n*, 808 F.2d 1471, 1 USPQ2d 1241 (Fed. Cir. 1986) (Claims to a process for making aramid fibers using a 98% solution of sulfuric acid were not anticipated by a reference which disclosed using sulfuric acid solution but which did not disclose using a 98% concentrated sulfuric acid solution.).

The rejection of claims 1, 4 and 9-15 under 35 U.S.C. 102(b) as being anticipated by Ekstrand et al. has also been carefully considered but is most respectfully traversed. Neither of the references meet the criteria for a proper rejection under 35 USC 102(b) as discussed above and as would be appreciated by one of ordinary skill in the art to which the invention pertains. This is admitted on page 6 of the

Official Action wherein it is indicated that the references relied upon in the anticipation rejections do not disclose the methylimidazole coupling agent which is a claim limitation and cannot be ignored as stated in MPEP section 2113. This section states that the structure implied by the process steps (here the methylimidazole coupling agent) should be considered when assessing the patentability of product-by-process claims over the prior art, especially where the produce can only be defined by the process steps by which the product is made. This is the structure implied by the steps of the process as noted in MPEP § 2113. For this reason alone, these rejections should be withdrawn.

Moreover, Ekstrand et al. describes the construction and properties of a sensitive RT assay, which it is fair to say, uses the same basic concept as the invention. This assay was the state of art when the work with the current invention started.

Shao et al. describes the application of this technology for determination of the RT inhibiting capacity of RT inhibitors and its use for evaluation of their mode of action. This document does not teach any significant improvements of the technique, but refer to the use of the methods described by Ekstrand et al. Shao does further not mention the procedure used for production of the crucial prA coated microtiter plates. This is only commented by Ekstrand et al., which describes the most relevant prior art.

Applicants are aware that the basic concept of the current invention is similar to prior art as described in the documents mentioned above. The current invention, however, provided the crucial innovations for application of this technique for commercial production of RT activity kits. One critical component, which distinguishes the current invention from prior art, is the microtiter plate. Ekstrand et al. does not describe the procedure for production of prA coated microtiter plates, but provides a reference to Suzuki et al. (J.Viol, Meth 1993, 44 p 189-198). The procedure described on page 191 in this document is based on the use of a combination of hydroxysulfosuccinimide and carbodiimide hydrochloride (EDC). Both chemicals are relatively expensive and the procedure is dependent on incubation with prA for 14-18 hours. At least hydroxysulfosuccinimide is sensitive to temperature and moisture and requires special precautions during storage.

In addition, the high reactivity of the reagents used makes it likely that they in addition to perform the intended coupling reaction also to some extent modify the bases in the prA polymer and thereby affecting the polymers ability to serve as template in the polymerase reaction. The plates further require storage in presence of prA and the coupling reagents until needed, and a wash just prior to use.

This makes batch quality control practically impossible! An additional drawback of this method is that the quality of the plates produced fluctuates. A variable proportion of the immobilized prA is according to our experience bound electrostatically. As a result the amount of product recovered after a typical RT reaction is affected by the washing procedures and may vary within and between different plate batches.

The procedure described might be useful for in-house production of research reagents, but not for commercial purposes. The new procedure according to the invention provides a simple, non-toxic; inexpensive method to manufacture large batches prA coated microtiter plates. The new plates are stable during storage, give minimal batch variation and can be delivered ready to use (without requirement of any extra wash prior to use).

Summing up, the procedure described in the current application is not an alternative way of producing prA coated microtiter plates, but a way to produce prA plates with new properties. These properties differs in several crucial aspects from those in prior art as described and/or used by Ekstrand et al., Shao et al. and Suzuki et al. Accordingly, it is most respectfully requested that these rejections be withdrawn.

The rejections of claims 1-4 and 9-15 under 35 U.S.C. 103 as being unpatentable over Shao et al., Ekstrand et al., Suzuki et al. and Rasmussen et al. has been carefully considered but is most respectfully traversed for the above reasons and reasons already of record and repeated below.

Applicants again wish to direct the Examiner's attention to the basic requirements of a prima facie case of obviousness as set forth in the MPEP § 2143. This section states that to establish a prima facie case of obviousness, three basic criteria first must be met. First, there must be some suggestion or motivation, either in

the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine the reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

Section 2143.03 states that all claim limitations must be taught or suggested by the prior art. In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). "All words in a claim must be considered in judging the patentability of that claim against the prior art." In re Wilson, 424 F.2d 1382, 1385, 165 USPQ 494, 496 (CCPA 1970). If an independent claim is nonobvious under 35 U.S.C. 103, then any claim depending therefrom is nonobvious. In re Fine, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988).

Applicants also most respectfully direct the Examiner's attention to MPEP § 2144.08 (page 2100-114) wherein it is stated that Office personnel should consider all rebuttal argument and evidence presented by applicant and the citation of In re Soni for error in not considering evidence presented in the specification.

The Examiner further argues that claims 1-4 and 9-15 are obvious when combining the teachings of Ekstrand et al., Shao et al., Suzuki et al. and Rasmussen et al. The three first documents and their relation to the current invention have already been discussed above. The addition of Rasmussen et al. to the panel is clever and takes us to the heart of the matter but is believed to be based on hindsight reconstruction based on Applicants' specification which is impermissible. In re Fritch, 23 USPQ 1780, 1784(Fed Cir. 1992) ("It is impermissible to engage in hindsight reconstruction of the claimed invention, using the applicant's structure as a template and selecting elements from references to fill the gaps.).

Applicants note that a citation from the Examiner's report "Suzuki et al. utilized Covalink plates from NUNC and the coupling agent EDC [1-ethy-3-(3-dimethylaminopropyl)-carboiimide] in N-hydroxysulfosuccinimide to bind the poly A to

the bottom of the plate. Rasmussen et al. utilizes the CovaLink NH plates in conjunction with the coupling agent EDC dissolved in I-methylimidazole. Single stranded DNA has the similar structure to poly dA and poly A, therefore, one of ordinary skill in the art would have had a high expectation of success in applying the EDC coupling agent in I-methylimidazole for the efficient directional binding to the bottom of the plate as taught by Rasmussen et al.". Although, this is what might be expected but only based on Applicants teaching. However, what really happened was that Applicants' attempts to bind prA according to the teachings of Rasmussen and all resulted in plates having bound prA with impaired function as template for reverse transcription! The reason for this was not evaluated in detail, but the results indicate that the method according to Rasmussen et al. is less specific for binding of RNA than of DNA and that the prA was bound also at other positions than at the 5' phosphate group. The reactivity of the EDC makes it also likely that it in addition to perform the intended coupling reaction also to some extent modify the bases in the prA polymer.

Further experiments revealed that the effect of EDC was clearly harmful and that the optimal immobilized prA template was achieved with 1-methylimidazole only. The efficiency of this binding was a function of both pH and imidazole concentration. The procedure was found useful for coupling of prA to CovaLink pates, Nucleolink strips and Nucelolink plates. Further, it was not useful for immobilization of ssDNA primers, which preferentially are immobilized according to Rasmussen et al. The prA bound according to our procedure is not affected by a wash of the plate in 1 M NaCl with 0.2% SDS, which efficiently removes prA unspecifically adsorbed to the plastic.

The prA coated microtiter plates according to the invention are thus superior to prior art as described by Ekstrand et al. and Suzuki et al. or to the corresponding plates produced according to the teachings of Rasmussen et al. A major benefit is the drastically increased assay sensitivity that is achieved with the new plates (demonstrated in table 5 and in examples 1-4). This unexpected effect is partially due to binding of increased amounts of prA according to the new procedure, but also to qualitative differences in the capacity of the bound prA polymer to serve as template for

reverse transcription. Applicants do not know what the structural differences between the prA microtiter plates are. From the literature can be extracted that 1-methylimidazole catalyses the formation of certain organic esters. The exact nature of the prA binding according to the invention is, however, not know, as the procedure does not correspond to methods recommended, or suggested by the manufacturer, or explicitly stated in the literature.

In summary, the new concept for production of prA plates is instrumental for large-scale production of prA coated microtiter plates and drastically improves the detection sensitivity of the RT assays. The assay kit of the invention provides a solid fundament for a new generation of RT activity kit assays. Accordingly, it is most respectfully requested that this rejection be withdrawn.

In view of the above comments and further amendments to the drawings and claims, favorable reconsideration and allowance of all of the claims now present in the application are most respectfully requested.

Respectfully submitted,

BACON & THOMAS, PLLC

By: Richard E Fichter

Richard E. Fichter
Registration No. 26,382

625 Slaters Lane, 4th Fl.
Alexandria, Virginia 22314
Phone: (703) 683-0500
Facsimile: (703) 683-1080

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